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Aptamers for Diagnostics with Applications for Infectious Diseases

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Keywords
aptamer, SELEX, biosensors, aptasensors, diagnostics, infectious diseases

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Chapter

Aptamers for Diagnostics with Applications for Infectious Diseases

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Abstract

Aptamers are in vitro selected oligonucleotides (DNA, RNA, oligos with modified nucleotides) that can have high affinity and specificity for a broad range of potential targets with high affinity and specificity. Here we focus on their applications as biosensors in the diagnostic field, although they can also be used as therapeutic agents. A small number of peptide aptamers have also been identified. In analytical settings, aptamers have the potential to extend the limit of current techniques as they offer many advantages over antibodies and can be used for real-time biomarker detection, cancer clinical testing, and detection of infectious microorganisms and viruses. Once optimized and validated, aptasensor technologies are expected to be highly beneficial to clinicians by providing a larger range and more rapid output of diagnostic readings than current technologies and support personalized medicine and faster implementation of optimal treatments.

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1. Introduction

In 1868, a young Swiss physician Friedrich Miescher isolated a new biological compound from nuclei of white blood cells, which had not been described before. He named it "nuclein" [1]. Today, it is known as "deoxyribonucleic acid (DNA)" which is nucleic acid in nature and carries heritable information for biological organisms. However, with advancements in the molecular genetics field, scientists started to discover new functions of nucleic acids other than storing and transferring genetic information [2].

In the 1980s, research on human immunodeficiency virus (HIV) and adenoviruses revealed a new understanding of the importance of selective interactions between nucleic acids and proteins. These studies demonstrated that viruses express small RNAs, which bind to cellular or viral proteins with high specificity. In parallel with these discoveries, scientists focused on deciphering fundamental features of short RNAs that can fold into unique three-dimensional structures [3]. In 1990, three separate groups [4–6] invented the systematic evolution of ligands by exponential enrichment (SELEX) method by which they obtained nucleic acid molecules, similar to naturally occurring nucleic acids, which have high specificities and affinities toward their targets. They named these in vitro selected molecules "aptamers." Since then, researchers have selected numerous aptamers against targets varying from small molecules to cells using SELEX [3].

1.1 Systematic evolution of ligands by exponential enrichment

SELEX is a technique to isolate an aptamer that is specific for the desired target from a randomized oligonucleotide (oligo) library by simulating evolution (systematic evolution of ligands). This in vitro technique includes a number of selection rounds (between 5 and 20) alternated with exponential amplification of the fittest oligonucleotides by PCR for DNA libraries or RT-PCR for RNA libraries (exponential enrichment).

In a typical SELEX experiment, the starting pool contains up to 10^{15} random oligonucleotide sequences. These sequences in the pool have a unique three-dimensional (3D) structures defined by the combination of interactions that include base pairing, stacking, sugar packing, and noncanonical intramolecular interactions. This structural complexity in the pool establishes a high probability of selecting an oligo that can interact avidly and specifically with the target of interest (aptamer). The intermolecular interaction between aptamer and target may include hydrogen bonds, salt bridges, van der Waals, and hydrophobic and electrostatic interactions.

Traditionally, SELEX is comprised of three main steps: incubation, separation, and amplification. The process involves incubating a "library" of oligos with randomized internal sequences of 20–60 nt with target molecules for a chosen period of time. Removal of unbound oligos from this mixture completes this initial step. Oligos that remain bound to the target are separated and amplified either by PCR or RT-PCR depending on the oligo type, DNA, or RNA. For DNA SELEX, biotin-labeled primers can be used in PCR, and the resulting double-stranded forms are separated using methods such as streptavidin bead capture. For RNA, T7 RNA polymerase promoter-containing primers are used in RT-PCR after which RNA libraries are amplified by in vitro transcription. The protocol follows the same amplification steps for several rounds (4–20) as for DNA SELEX (**Figure 1**). Negative selection can be included in either RNA or DNA SELEX protocols, which is achieved by passing the nucleic acid pool over a supporting matrix in the absence of the target. This step aims at eliminating the oligos that bind the matrix in a target-independent manner. Analogs of the target can also be included during selection rounds as competitors of binding if there is a means of separating the target from the analog competitors. This competition is expected to result in aptamers with higher specificity for the target over the analogs.

Two alternative protocols are followed after these rounds to complete the SELEX. The selected oligos from the final round are cloned and sequenced for aptamer identification. Alternatively, high-throughput sequencing (or next-generation sequencing) can be employed to obtain sequence data from oligos present in the pool after different rounds of selection. Comparative sequence analysis allows pinpointing

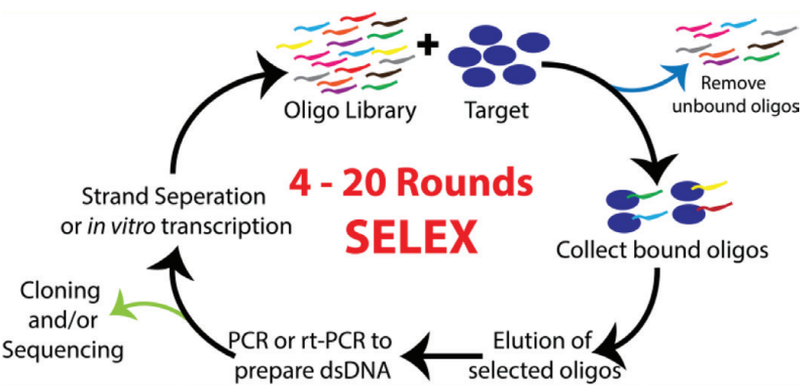

Figure 1.
Schematic representation of SELEX protocol.

consensus sites that are potentially involved in target recognition [7, 8]. The most promising sequences are synthesized and characterized further, among which aptamers with nanomolar dissociation constants are frequently identified. In some instances, aptamers have also been isolated with picomolar dissociation constants.

In vitro selection can take months. To shorten the time for selecting high affinity aptamers, Golden et al. suggested a new method for SELEX: photochemical SELEX (PhotoSELEX) [9]. The technique involves the evolution of modified DNA aptamers, which are capable of forming a photoinduced covalent bond with their targets. Thus, these aptamers have greater specificity, and fewer selection rounds are required to select aptamers compared with the traditional SELEX methodologies.

1.2 Aptamer structure

Aptamer selection directly depends on environmental components during SELEX. This is because the ionic components and pH of the environment can dictate the predominant structures of oligos in the pool. Nucleic acids are negatively charged molecules that create an "ionic atmosphere" for ion-nucleic acid interactions, a freely joined sheath of ions surrounding the nucleic acids. These electrostatic interactions directly affect the structure of nucleic acids and thus the target binding by changing charge distribution. For specific ligand binding, it is crucial that the aptamer reliably forms the appropriate three-dimensional (3D) structure. However, structures of short oligonucleotides like aptamers are affected by the incubation temperature and the components of the operating buffer system such as the specific ions, ionic strength, and pH. Therefore, the affinities and future performance of aptamers depend on the buffers used during aptamer selection, and the choice of buffer present during selection requires attention when developing a SELEX protocol [10–13].

Some factors present in the samples to be analyzed may have a negative impact on aptamer performance. For example, aptamers are susceptible to nucleases that are present in many biological samples. This is particularly true for RNA, having the 2'OH group, which can electrophilically attack the phosphate of the nucleic acid backbone. Nucleases promote this chemical property to catalyze hydrolysis of RNA, and this property also makes RNA more chemically labile to high pH and temperature compared with DNA. To counter this susceptibility to hydrolysis and to stabilize nucleic acids, many post-selection chemical modifications can be made. However, incorporating these modifications into the aptamer after its selection carries a large risk of altering the aptamer structure with a resulting loss of affinity for the target analyte. Alternatively, the less risky approach is to use chemically substituted nucleotide analogs during SELEX.

Secondary motifs in the tertiary structures of aptamers are diverse. Such motifs include the "stem-loop," "hairpin structure," "pseudoknot," "internal bulge," "kissing loop," "three-way junction," and "the G-quadruplex." To understand these structures in detail, X-ray crystallography or nuclear magnetic resonance spectroscopy is utilized. But these techniques are laborious and expensive. To ease the process, computer algorithms have been developed to estimate the lowest free-energy structures using sequence-based modeling [14]. This makes it possible to quickly predict the secondary structure of oligonucleotides without needing many resources. However, computational approaches to obtaining 3D models of nucleic acid structures by modeling from primary sequence are still in the development phase, and the results are not as reliable as experimental methods [15–17].

Aptamers have a significant advantage over antibodies as components of sensing units. One advantage is that nucleic acid structures can be regenerated several times with little activity loss, whereas protein-based antibodies can only be used once or a few times before their functionality is lost. In contrast to antibodies or enzymes,

Features	Aptamers	Antibodies
Target Molecules	Any molecule and cell	Limited to immune response producing targets
Molecular Structure	Nucleic acid or peptide	Protein
Size	Small (<30 kDa)	Large (>75 kDA)
Stability	Reversible after rounds of denaturation	Easily lose function after denaturation
Chemical Functionality	Easy to modify chemical structure	Hard to modify chemical structure
Immunogenicity	Non-immunogenic	Immunogenic
Affinity & Specificity	High	High
Generation Time	Several Weeks	Several Months
Cost	<$50 / gram	~$300 / gram
Storage	Stable at room temperature; No need to freeze	Easily denaturated; Need to freeze
Shelf Life	Several years (if frozen or dried)	~6 months

Table 1.
Comparison of aptamers with antibodies.

nucleic acid aptamers are often highly stable and can be inexpensively synthesized with high reproducibility and purity. Like antibodies, they bind their targets with high affinity and specificity (**Table 1**). These properties are motivating the current flood of reports of aptamer-based biosensors employing a wide range of technologies.

1.3 Aptamers for diagnostic and therapeutic applications

Development of novel biosensors for various clinical diseases has become essential as new health issues emerged. To meet this goal, antibodies have been used extensively; however, more recently, aptamers have been recognized as promising alternatives for developing diagnostic devices.

A biosensor is a tool with the ability to provide a measurable signal as a result of biomolecular interactions. Biosensors generally consist of two components: a bioreceptor and a transducer. The bioreceptor binds specifically to the molecule of interest, and the transducer turns information from the binding event into a detectable signal. The components of the transducer include a detector and a reporter, which acts as a bridge between the bioreceptor and the detector.

The most critical part of the biosensor is the bioreceptor (or bio-recognition element). The success of the sensor directly depends on its high affinity and specificity. Because of their more flexible structures, aptamers can provide a substantial signal in combination with a larger number of detection methods than possible for antibodies. Aptamers are preferred over antibodies for biosensor applications because of their cost, stability, and reusability as well as the aforementioned advantages (see **Table 1**). Biosensors, called "aptasensors," have been developed with many detectors including electrochemical, optical, microcantilever, and acoustic detectors [10].

Aptamers are also used in therapeutic applications, which will not be extensively discussed in this chapter. For discussions of therapeutic applications, the reader is directed to reviews that focus on this topic [18–21]. The majority of therapeutic aptamers inhibit their target molecules, and some act as receptor agonists. Potential therapeutic aptamers against proteins including nucleolin, chemokine ligand 12, or thrombin have been described. Several RNA and DNA aptamers are undergoing clinical trials, yet only pegaptanib against vascular endothelial growth factor has so far been in the USA by the FDA for the treatment of vascular ocular disease [3].

To advance therapeutic applications, drug delivery systems with aptamers have been developed. For such applications, aptamers are needed that recognize cell surface proteins, which can be challenging to select because these proteins are difficult to purify in their natural conformations. The development of cell-based selection techniques has enabled aptamer selection against proteins in their native form while they are on the cell surface. These selections are performed against single-cell types [22]

to obtain aptamers with the ability to bind cell surface proteins specifically expressed on the surface of the cell type used for selection. Delivery systems with these aptamers can carry a variety of cargos into cells by taking advantage of the surface protein internalization in response to aptamer-receptor binding.

2. Aptamer use in diagnostics

Diagnostics is one of the most dynamic fields in biosensor research. To support early diagnosis and individualized medicine, researchers seek to develop methods to detect identified biomarkers by more sensitive, time-saving, and cheaper methods. The perfect sensor for medical diagnostics should also be specific, reusable, easy to monitor, nonreactive, and stable with various biological samples. Aptamers can be developed to meet all these requirements. With their wide spectrum of possible targets, sensitive detection of virtually all toxins, drugs, peptides, proteins, metabolites, biomarkers, and cells is possible with aptamers.

Several types of aptasensors have been developed based on electrochemical, optical, mechanical, and acoustic approaches. An early use of aptamers as bio-recognition elements in aptasensors was reported in 1996 with an optical biosensor that utilized fluorescently labeled aptamers in a homogenous assay [23]. Later, aptamers were integrated onto solid supports, which provided an opportunity for real-time analyte detection. Most of these studies have been at the proof-of-principle level, and the majority of studies have been performed with the thrombin aptamer (TA), which has the advantage of a stable target and aptamer. Thrombin aptamer self-assembles into a highly stable G-quadruplex structure, and thrombin is a structurally stable globular protein found in blood. Future studies to optimize other aptasensors with less stable aptamers and analytes in complex matrices are likely to be challenging.

Aptamers fold their flexible, single-stranded chains into 3D structures, which may change upon binding to their cognate target molecules. This structure-switching characteristic has been capitalized on in many aptamer applications. An early example used electrochemical sensing with aptamers immobilized on an electrode surface, and target binding is observed by measuring electrochemical current variations. This system utilized an amperometric sandwich assay combining TA on a gold electrode which was used to capture the TA-labeled glucose dehydrogenase (GDH) [24].

Several types of aptasensors have been developed based on electrochemical, optical, mechanical, and acoustic approaches, which are discussed in the following sections.

2.1 Electrochemical aptasensors

Electrochemical aptasensors are constructed by attaching an aptamer that carries a redox-active moiety to an electrode surface. Such aptasensors can make use of voltammetric (amperometric), potentiometric, conductometric, or impedimetric assays for analyte detection. In some formats, the aptamer is labeled with an electroactive group and a structural change in the aptamer upon binding to the target analyte and changes the distance of the electroactive group from the electrode surface, resulting in the switching "on/off" of the electrochemical signal. Measurement of changes in electrochemical features after target binding has been used to determine target concentration [24]. So far, electrochemical aptasensors have been reported for a wide range of targets including PDGF, thrombin and immunoglobulin E (IgE), cocaine, theophylline, adenosine, aminoglycosides, and adenosine triphosphate (ATP) and inorganic ions such as potassium (K^+) [10].

In addition to their innate structural changes on binding their target molecules, other structural constraints can be applied to aptamers that result in signals for detection by electrochemical approaches. The most commonly used additional constraint is an oligonucleotide (either connected with the aptamer or separate) that is complementary to part of the aptamer and, upon hybridizing with the aptamer, constrains its structure to an inactive form. For example, a biotin-tagged DNA aptamer for zeatin was hybridized with a complimentary "assist DNA" to form a Y-type DNA structure. Avidin-modified alkaline phosphatase (ALP) was attached to this structure with two biotins at the terminals of DNA aptamer. In the presence of zeatin, this complex was disrupted which leads to a decrease in the oxidation signal from p-nitrophenol (PNP) produced by the catalytic effect of ALP. From this, zeatin concentration in the range of 50 pM–50 nM was selectively measured with a detection limit of 16.6 pM [25].

Redox-active methylene blue (MB) has been used as an aptamer label and electrotransfer communication agent with the electrode. Methylene blue enables the detection of changes in aptamer conformation upon target binding. For example, an MB-labeled TA was used to construct an aptasensor by its immobilization on an electrode. The flexible conformation of the aptamer enabled the electrotransfer from MB to the electrode. The structural change upon analyte binding shielded MB in a "signal-off" mode. However, this mode is a disadvantage for diagnostics because the amperometric response decreases as a result of the association of the target thrombin with the aptamer.

Several approaches have been taken to develop aptasensors that operate in a "signal-on" mode. As an example, the TA was modified with an electroactive ferrocene group as the redox label at one end and a thiol group at the other end. The electrical contact of the electrode with the ferrocene label was affected by the long, flexible aptamer chain. Thrombin binding stabilizes the aptamer's G-quadruplex conformation, which brings the ferrocene group closer to the electrode. This close proximity enables electron transfer between the electro-active ferrocene units and the electrode, thus producing a positive signal in the presence of thrombin. A similar approach to creating signal-on electrochemical aptasensors utilized the conformational change in the cocaine aptamer that occurs on binding its target. In the absence of the target, the aptamer on the solid surface stays in a partially folded form as a three-way junction. Cocaine binding decreases the distance for electron transfer and thus increases the signal [26].

Demirkol et al. generated an electrochemical aptasensor to detect *E. coli* O157:H7. The electrode surfaces were modified by cysteamine via self-assembled monolayer formation. The carboxyl-functionalized quantum dots and aptamers were conjugated to cysteamine-modified gold electrodes [27]. Ge et al. reported an affinity-mediated homogeneous electrochemical aptasensor using graphene-modified glassy carbon electrode (GCE) as the sensing platform. In this approach, the aptamer-target recognition is converted into an ultrasensitive electrochemical signal output with the aid of a novel T7 exonuclease (T7Exo)-assisted target-analog recycling amplification strategy, in which ingeniously designed methylene blue (MB)-labeled hairpin DNA reporters are digested in the presence of target and, then, converted to numerous MB-labeled long ssDNAs. The distinct difference in differential pulse voltammetry response between the designed hairpin reporters and the generated long ssDNAs on the graphene/GCE allows ultrasensitive detection of target biomolecules [28]. Lai et al. proposed a renewable electrochemical aptasensor for super sensitive Hg^{2+} determination [29]. The novel aptasensor, based on sulfur-nitrogen co-doped ordered mesoporous carbon (SN-OMC) and a thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) mismatch structure, used ferrocene as signal molecules to achieve the conversion of signal to current. In the absence of Hg^{2+},

the thiol-modified T-rich probe 1 spontaneously formed a hairpin structure by base pairing. After hybridizing with the ferrocene-labeled probe 2 in the presence of Hg^{2+}, the hairpin structure of probe 1 was opened due to the preferential formation of the T-Hg^{2+}-T mismatch structure, and the ferrocene signal molecules approached the modified electrode surface. Sulfur-nitrogen co-doped ordered mesoporous carbon with high specific surface area and ample active sites acted as a signal amplification element in electrochemical sensing. The sensitive determination of Hg^{2+} can be actualized by analyzing the relationship between the change of oxidation current caused by ferrocene and the Hg^{2+} concentrations [29]. Finally, Wang et al. combined the strengths of advanced aptamer technology, DNA-based nanostructure, and portable electrochemical devices to develop a nanotetrahedron (NTH)-assisted aptasensor for direct capture and detection of hepatocellular exosomes. The oriented immobilization of aptamers significantly improved their accessibility to suspended exosomes, and the NTH-assisted aptasensor could detect exosomes with 100-fold higher sensitivity when compared to the single-stranded aptamer-functionalized aptasensor [30].

Recently, nanoporous metal surfaces have been found as good sensor platforms for aptamers. Nanoporous gold [31, 32]-based sensors have been used with the TA and ATP aptamers and a redox probe to provide the electrons to the gold surface for sensitive detection of analyte by electrochemical impedance spectroscopy (EIS). The ATP aptamer was in a split format with the second half of the aptamer covalently linked with 3,4-diaminobenzoic acid (DABA), which created the EIS signal by its oxidation at the gold interface. For an analyte that can undergo redox reactions, such as bisphenol A (BPA), this property can be used to provide a signal [33]. Other nanoporous surfaces such as graphene oxide/Au composites and porous PtFe or PtTiAl ternary alloys have also been employed to measure breast cancer cells using the MUC-1 aptamer linked with the electroactive label thionine [34] or kanamycin with $[Fe(CN)6]^{3-/4-}$ [35]. The ability of aptamers to hybridize with other oligonucleotides was employed to create molecular gates over the pores in nanoporous gold surfaces. The gate, created with an aptamer highly specific for the avian influenza viruses (AIV) H5N1 hybridized to oligos linked to the nanoporous gold surface, was closed in the absence of AIV H5N1 but open when the virus bound and released the aptamer. The open pores allowed the entry of substrate and cofactor for lactate dehydrogenase, layered on a glassy electrode below the nanoporous gold. Cyclic voltammetry was used to detect the gold-catalyzed oxidation of the NADH produced as a result of LDH activity [34].

Nanoporous anodized aluminum oxide surfaces have more recently been used for providing a nanoporous surface through which electron movement can be controlled by aptamer-analyte binding. In these aptasensors, the aptamers are attached to a gold surface, which is provided by 2 nm gold nanoparticles [36] or by a surface coating created by sputtering [37]. The structural change in the aptamer due to binding of the analyte reduces access of $[Fe(CN)6]^{3-/4-}$ to the gold surface [36], which can be measured by EIS. Even in the absence of a redox probe, a good EIS signal can be obtained due to a combination of steric hindrance and change in electrical conductance around the pores resulting from the structural changes that occur in the highly negatively charged aptamers upon binding their targets [37].

2.2 Optical aptasensors

Another type of biosensor that utilizes aptamers as bio-recognition elements is the optical sensor, for which fluorescent and colorimetric assays are the two widely used formats. In general, fluorescent detection is preferred due to its suitability for real-time detection and because there are many available labeling options as fluorophores and quenchers, which can easily be incorporated during aptamer synthesis.

To convert aptamers into fluorescent signaling probes, several strategies have been developed. A frequently used format places an aptamer sequence in a molecular beacon-like, hairpin structure in which ends are labeled either with two fluorophores or a fluorophore and a quencher. This system utilizes Förster resonance energy transfer (FRET), which relies on the energy transfer between donor and acceptor. Upon target binding, the structure is disrupted by separating the two ends, thus leading to a fluorescence signal. In this format, the use of organic fluorescent dyes or quantum dots (QDs) improved the assay performance and could also be used to detect drug delivery in cells. Another format places a fluorophore-labeled aptamer in a duplex structure with a complementary DNA sequence labeled with a quencher. The aptamer target successfully competes with the complementary DNA resulting in departure of the complementary strand from the aptamer and an accompanying increase in the fluorescence signal [26].

In optical analysis, simultaneous detection of several analytes is readily achieved by multiplexing. In one of the earliest examples, fluorescently labeled aptamers were immobilized on a glass surface. In this system, detection of thrombin and three cancer-biomarker proteins, inosine monophosphate dehydrogenase, vascular endothelial growth factor, and basic fibroblast growth factor, was achieved by fluorescence polarization even in the presence of human serum and *E. coli* cell lysates [10]. The use of aptamer-linked beads in a microarray setting brought a further sophistication of an "electronic tongue" that consists of a fluid delivery system and a fluorescence microscope attached to a digital camera for quantification [10].

Graphene oxide-based aptasensors can also be readily multiplexed [10]. As an example, a novel label-free fluorescent approach was constructed for H1N1 detection based on graphene-oxide and strand displacement reaction, using SYBR Green I (SGI) for signal amplification [38]. Another example is an assay for detection of the pathogenic bacterium, *Pseudomonas aeruginosa*. This assay was enabled by highly specific aptamers conjugated with photoluminescent carbon dots as the fluorescent probe and graphene oxide as the quencher, and it allowed detection of as low as 9 CFU mL^{-1} *P. aeruginosa* [39]. Electrochemiluminescence is another output option for aptasensors [40].

2.3 Microcantilever aptasensors

Incorporating aptamers into microcantilever sensors offers the possibility of label-free target detection, low noise, high scalability, and small testing volumes [10]. Microcantilever-based sensing has been incorporated into several experimental chemical and biological sensing systems due to its small size, low cost, low sample volume, label-free detection, and ease of integration with microfluidic devices [41]. High-throughput analysis is achievable via microcantilever arrays for parallel processing, although they cannot easily be extensively multiplexed. Microcantilever aptasensors can be operated in either static or dynamic mode. In a liquid environment, the static mode can be more sensitive compared to the dynamic mode. In the static mode, one side of the microcantilever is functionalized with aptamers for analyte detection. Surface stress is generated when target analyte adsorbs onto the functionalized surface. The difference in surface stress between the top and bottom surface results in microcantilever bending, which can be upward (positive) or downward (negative) depending on the type of molecular interactions involved. Displacement of the beam can be detected by using readout techniques such as optical, piezoresistive, and capacitive. The optical technique is the most popular approach because it has high resolution and linear response and produces absolute displacement measurement. Detection of proteins and small molecule analytes, with an aptamer-decorated cantilever, can be achieved with many sensing means including interferometry [42–44] and piezoresistivity [45, 46]. Further

development of microcantilever devices will require solutions to their sensitivity to vibration and the limits to which they can be multiplexed.

2.4 Acoustic aptasensors

Early work on acoustic aptasensors included the modification of gold-coated quartz crystals with aptamers. Target binding changes the frequency or phase shift which can be detected as a change in the input and output light. For example, the IgE DNA aptamer on a quartz crystal microbalance (QCM) format provided a detection limit of 3.3 $ng.cm^{-2}$ of IgE [47].

Label-free and real-time quantification of proteins were also measured by the propagation of the acoustic wave in a surface acoustic wave (SAW) biosensor, which included an array of five sensor elements to detect human α-thrombin or HIV-1 Rev peptide. This system had a detection limit of 75 $pg.cm^{-2}$ for both α-thrombin and HIV-1 Rev peptide as analytes [48]. The aptamers demonstrated a better linear response, stability, and reusability when compared with antibodies specific for IgE.

Surface plasmon resonance (SPR) sensors, similar to QCM and SAW aptasensors, rely on a change in refractive index due to target binding. Quartz crystal microbalance and surface plasmon resonance aptasensors for detection of HIV-1 Tat protein were found to have similar high specificities with the SPR sensor having a wider linear range [49]. The SPR aptasensor for retinol binding protein-4 was found more sensitive than an ELISA [50]. In developing SPR aptasensors, combined approaches resulted in a microfluidic device with interdigitated transducer creating high-frequency acoustic waves for target separation [51]. Biotinylated-thrombin aptamers were captured by streptavidin-functionalized polystyrene, which was pumped in the microchannel after incubating with sample. In the microfluidic device, SAW exposure leads to separation of thrombin captured in the polystyrene by its aptamer from the nontarget serum proteins.

Gold nanoparticles (AuNPs) have also been utilized in developing SPR aptasensors. A U-shaped fiber-optic SPR biosensor was developed for the rapid detection of BPA [52]. Incubation of bare AuNPs with BPA aptamer resulted in AuNPs/ssDNA complexes which are stable in high salt. Bisphenol A binding disrupted this complex, which resulted in the aggregation of AuNPs and enhanced refractive index of the solution in the fiber-optic SPR sensor. This system had a detection limit of 3.7 $pg.mL^{-1}$, and linear range was 0.01–50 $ng.mL^{-1}$.

Neves et al. generated two sensitive cocaine aptasensors that rely on an electromagnetic piezoelectric acoustic sensor (EMPAS) platform as the basis of ultra-high frequency with tuned signal-to-noise ratio [53]. The sensing interface consists of a S-(11-trichlorosilyl-undecanyl) benzenethiosulfonate (BTS) adlayer-coated quartz disc onto which a structure-switching cocaine aptamer was immobilized, completing the preparation of the MN4 cocaine aptamer with an apparent Kd of 45 ± 12 μM and limit of detection of 0.9 μM. The same group developed an MN6 cocaine aptasensor using an EMPAS platform that had apparent Kd of 27 ± 6 and a 0.3 μM detection limit [54].

Detection of cells using SPRs has some limitations that have been creatively overcome. The first limitation includes nonselective binding that causes the refractive index changes, which can be circumvented by reference flow cells to offset this effect [55]. Second is the sensing range, which is typically around 200 nm compared with cell dimensions that are in the micron range. Using long-range SPR, the depth was increased over 800 nm, which increased the sensitivity for cell detection [56]. Another drawback is its low-throughput, which has been resolved by SPR imaging technology.

3. Clinical perspective

As discussed throughout this chapter, aptamers can be evolved to have high affinity and specificity for a range of target molecules that includes small organics, peptides, protein, sugar, viruses, bacteria, parasites, live cell, and tissue (**Tables 2** and **3**). This characteristic paves the way for aptamers to have applications in various disciplines including sensing, medicine, pharmacology, and microbiology. Aptamer-based sensors have great promise as effective tools in the areas of diagnostics and therapeutics for clinical use [164, 165].

3.1 Clinical diagnostics

In clinical practice, the quantification of single biomarkers is frequently not sufficient to support a confident diagnosis. Thus, multiple analyses are required for each diagnosis, many of which might rely on antibodies, the current workhorses of the diagnostic world. Although many antibodies are highly sensitive and specific for their antigen targets, they often suffer from batch-to-batch variation. ELISA assays, which are the main diagnostic platform for antibodies, have been improved with protocols that increase their efficiency and the specificity of their output. However, ELISA assays cannot be readily multiplexed to measure simultaneously the many biomarkers required for a confident diagnosis. A strength of aptamer biosensors is their ability to be multiplexed. Another strength is the range of technologies that can be applied to produce operating aptasensors [166–168]. With these strengths to drive it, aptasensor technology is one of the fastest growing biotechnology areas in diagnostics with an expectation of reaching about $250 million by 2020 [169, 170].

Aptamers can provide new opportunities for medical diagnostics beyond what is available with antibodies [18, 171]. For example, aptamers can be selected against non-immunogenic and toxic targets, to which antibodies cannot be elicited. These short, single-stranded oligonucleotides can be synthesized via simple chemical synthesis, making them easier and less costly to produce than antibodies [172]. When synthesized in cells containing DNAs encoding RNA aptamers, they can fold appropriately and recognize intracellular targets [173–175].

A limited number of aptasensors are in the pipeline to be used in the areas such as biomarker and microorganism detection and cancer clinical testing. The available aptasensors include (1) OTA-Sense for detection of Ochratoxin A (OTA), a toxin produced by fungi [165, 176]; (2) AflaSense for detection of aflatoxins [95, 177–179]; (3) AptoCyto for flow cytometry applications [165]; (4) AptoPrep as a kit including conjugated aptamers specific to CD-31, EGFR, HGFR, and ICAM-2 [165]; (5) SOMAscan as a platform with the ability to detect >1300 proteins from small volumes [180–184]; (6) an aptamer-based proteomics technology developed by Jung et al. for detecting non-small cell lung cancer [165]; and (7) OLIGOBIND for measuring the thrombin level in blood [185].

Highly sensitive tests are required to specifically detect cancer cells in body fluids over all others. Success in this effort requires the identification of biomarkers that are found only on tumor cells. Some aptamers have been identified that might be applied to detecting tumor cells in the blood. For example, cancer cells and normal cells have been distinguished by using electrochemical sensors, a SERS active bimetallic core-satellite nanostructure, porphyrin-based covalent organic framework based aptasensor [186], and a deterministic lateral displacement (DLD) pattern-based aptamer-tailed octopus chip [187]. Recently, some aptasensors have been tested in cancer studies. Prostate-specific antigen (PSA), mucin 1 (MUC1), PDGF-BB, and vascular endothelial growth factor (VEGF) are detected as cancer biomarkers in cancer cell lines [165, 188].

Organism	Target	Aptamer	Backbone	Binding affinity (Kd)	Reference
M. tb Beijing strain	ManLAM	T9	DNA	668 ± 59 nM	[57]
M. tb H37Rv	CE protein	CE24	DNA	0.375 μM	[58]
	CE protein	CE15	DNA	0.16 μM	
M. tb	CE protein	CSIR 2.11	DNA		[59]
	MPT64	MPT64-A1	DNA		[60, 61]
	EsxG protein	G43	RNA	8.04 ± 1.90 nM	[62]
	EsxG protein	G78	RNA	78.85 ± 9.40 nM	
	Whole bacterium	MA1	DNA	12.02 nM	[63]
	Whole bacterium	Aptamer 1	DNA	37 ± 4 nM	[64]
S. typhimurium	Outer membrane proteins	Aptamers 33 and 45	DNA		[65, 66]
	OmpC protein	I-2	RNA	20 nM	[67]
	Whole bacterium	C4	DNA		[68]
	Whole bacterium	ST2P	DNA	6.33 ± 0.58 nM	[69]
	Whole bacterium	SAL 26	DNA	123 ± 23 nM	[70]
Salmonella Paratyphi A	Whole bacterium	Apt22	DNA	47 ± 3 nM	[71]
S. enteritidis	Mixtures of 10 strains of *S. enteritidis*	S25	RNA		[72]
	Whole bacterium	crn1	DNA	0.971 μM	[73]
	Whole bacterium	crn2	DNA	0.309 μM	
S. aureus	S. Endotoxin B	$APT^{SEB}1$	DNA		[74]
	S. Endotoxin C1	C10	DNA	65.14 ± 11.64 $nM.L^{-1}$	[75]
	Alpha toxin	R12.06	DNA	93.7 ± 7.0 nM	[76]
	Peptidoglycan	Antibac1	DNA	0.415 ± 0.047 μM	[77]
	Peptidoglycan	Antibac2	DNA	1.261 ± 0.280 μM	
	Protein A	PA#2/8	DNA	172 ± 14 nM for the recombinant Protein A and 84 ± 5 nM for the native Protein A	[78]
	Whole bacterium	SA20	DNA	70.68 ± 39.22 nM	[79, 80]
	Whole bacterium	SA23	DNA	61.50 ± 22.43 nM	
	Whole bacterium	SA31	DNA	82.86 ± 33.20 nM	
	Whole bacterium	SA34	DNA	72.42 ± 35.23 nM	
	Whole bacterium	SA43	DNA	210.70 ± 135.91 nM	
	Whole bacterium	SA17	DNA	35 nM	[81]
	Whole bacterium	SA61	DNA	129 nM	
L. monocytogenes	Internalin A	A8	DNA		[82]
	Listeriolysin O	LLO-3	DNA		[83]
	Whole bacterium	Lbi-17	DNA		[84]
	Whole bacterium	LMCA2	DNA	$2.01x10^{-12}$ M	[85]
	Whole bacterium	LMCA26	DNA	$1.56x10^{-10}$ M	
E. coli 0157	LPS	E-5, E-11, E-12, E-16 to E-19	DNA		[86]
E. coli 0158	Whole bacterium	AM-6	DNA	107.6 ± 67.8 pmol	[87]
E. coli K88	Whole bacterium	Apt B12	DNA	15 ± 4 nM	[88]

Organism	Target	Aptamer	Backbone	Binding affinity (Kd)	Reference
E. coli KCTC 2571	Whole bacterium	E1	DNA	12.4 nM	[35, 89]
	Whole bacterium	E2	DNA	25.2 nM	
	Whole bacterium	E10	DNA	14.2 nM	
	Whole bacterium	E12	DNA	16.8 nM	
E. coli DH5α	Whole cell	Ec3(31)	RNA	225 nM	[90]
	Whole cell	8.28A	DNA	27.4 ± 18.7 nM	[91]
M. tb H37Rv	Whole bacterium	NK2	DNA		[92]
	ManLAM	ZXL1	DNA	436.3 ± 37.84 nM	[93]
BCG	ManLAM	BM2	DNA	8.59 ± 1 .23 nM	[94]
M. tb	Acetohydroxyacid synthase	Mtb-Apt1	DNA	1.06 ± 0.10 μM	[95]
	Acetohydroxyacid synthase	Mtb-Apt6	DNA	0.210 ± 0.05 μM	
S. typhi	Type IVB pili	S-PS8.4	RNA	8.56 nM	[96, 97]
S. aureus	SEA	S3	DNA	36.93 ± 7.29 nM	[98]
	α-toxin	AT-33	DNA		[99]
	α-toxin	AT-36	DNA		

Table 2.
Aptamers selected against bacteria.

Organism	Target	Aptamer	Backbone	Binding affinity (Kd)	Reference
HIV-1	Tat	RNA^{Tat}	RNA	120 ± 13 pM	[49, 100, 101]
HCV	E2 protein	ZE2	DNA	1.05 ± 1 nM	[102]
	Core protein	9–14	RNA	142 nM	[103]
	Core protein	9–15	RNA	224 nM	
	Core protein	C4	DNA		[104, 105]
Influenza A virus (H3N2)	HA(91–261)	A22	DNA		[106]
	HA	Clone B	RNA	200 pM	[107]
	Whole virus	P30-10-16	RNA	188 pM	[108]
Influenza A virus (H5N1)	HA	A10	DNA		[109]
Influenza A virus (H9N2)	HA(101–257)	C7	DNA		[110]
Influenza A virus (H5N1)	HA	HAS15–5	RNA		[111]
Influenza A virus (H1N1)	HA	D-26	RNA	67 fM	[112]
Influenza A virus (H3N2)	HA	HA68	DNA	7.1 nM	[113]
Influenza A virus (H5N1) and (H7N7)	HAs from H5N1 and H7N7	8-3	RNA	170 fM	[114]
Influenza A virus	HA1 subunit	ApI	DNA	64.76 ± 18.24 nM	[115]
Influenza A virus	HA1 subunit	ApII	DNA	69.06 ± 12.34 nM	
Influenza A virus	HA1 subunit	ApIII	DNA	50.32 ± 14.07 nM	

Organism	Target	Aptamer	Backbone	Binding affinity (Kd)	Reference
Influenza A virus (H9N2)	HA	A9	DNA	46.23 ± 5.46 nM	[116]
Influenza A virus (H9N2)	HA	B4	DNA	7.38 ± 1.09 nM	[117]
Influenza A virus (H5N1) and (H5N8)	Whole virus	IF22 and IF23	DNA		[118]
HBV	Surface antigen	HBs-A22	RNA		[119]
HPV 16	E7 protein	G5α3N.4	RNA	1.9 μM	[120]
HIV-1	Gp120	B40	RNA	21 ± 2 nM	[121]
	Gp120	B40t77	RNA	31 ± 2 nM	
	Gp120	A-1	RNA	52 nM	[122]
	Gp120	BclON-mut	DNA	143 ± 79 nM	[123]
	Gp120	F-thio-BclON	DNA	86 ± 17 nM	
	RT	1.1RNA	RNA	5 nM	[124]
	RT	RT1t49	DNA	1 nM	[125]
	RT	4.20	DNA	180 ± 70 pM	[126]
	RT	R12–2	DNA	70 nM	[127]
	RT	37NT	DNA	660 pM	[128]
	RT	FA1	FANA aptamer	Low pM range	[129]
	5'-UTR of HIV-1 genome	RNApt16	RNA	280 ± 60 nM	[130]
	TAR RNA element	IV04	DNA	20 nM	[131]
	Integrase	T30695	DNA	0.5 ± 0.2 μM	[132, 133]
	Integrase	93del	DNA		[56, 134, 135]
	Nucleocapsid protein	8–6	RNA	$1.4x10^{-9}$ M	[136]
	Gag protein	DP6–22	RNA	100 ± 3.4 nM	[137]
	Rev protein	RBE(apt)	RNA		[138]
	Integrase	S3R3	RNA	47 ± 3 nM	[139]
HCV	NS3 protein	G6–16	RNA	238 nM	[140]
	Truncated protease domain of NS3 protein	G9-I	RNA	10 nM	[141]
	Helicase domain of NS3	G5	RNA	25 nM	[142]
	IRES domains III-IV	3–07	RNA	9 nM	[143]
	IRES	AP50	RNA	5 nM	[144, 145]
	IRES domain IIIf and IV	HH-11	RNA		[146]
	NS5B	27v	DNA	132.3 ± 20 nM	[147, 148]
	NS5B	r10/43	RNA	1.3 ± 0.3 nM	[149]
	NS5B	r10/47	RNA	23.5 ± 6.7 nM	
	NS5B	R-F t2	RNA	2.62 ± 0.90 nM	[85, 150]

Organism	Target	Aptamer	Backbone	Binding affinity (Kd)	Reference
Influenza A virus (H5N2)	Glycosylated HA	HA12–16	RNA		[151]
Influenza A virus (H1N1, H5N1, H7N7 and H7N9)	Residues in the N-terminal of the PA_N of the influenza A virus polymerase	PAN-2	DNA	247 ± 11 nM	[152]
HBV	Truncated P protein	S9	RNA		[153]
	Core protein	Apt.No.28	DNA		[154]
	Capsid	AO-01	DNA	180 ± 82 nM	[155]
HPV 16	E7 protein	A2	RNA	107 nM	[156, 157]
	E6 protein	F2	RNA		[158]
SARS-CoV	Helicase	NG8	DNA	5 nM	[159]
DENV-2	Envelope protein domain III	S15	DNA	200 nM	[160]
RABV	Glycoprotein	GE54	DNA	307 nM	[161]
EBOV	EBOV sGP	39SGP1A	RNA	27 nM	[162]
Zika	NS1 protein	Clone 2	DNA	24 pM	[163]
	NS1 protein	Clone 10	DNA	134 nM	

Table 3.
Aptamers selected against viruses.

Many conventional diagnostic technologies for detecting virus and bacteria, including serologic-, nucleic acid-, and culture-based tests, are either time-consuming or expensive on account of the need for sophisticated equipment [188]. For example, the gold standard for laboratory diagnosis of acute viral infections is isolation and characterization of the virus or bacterium. Isolation and long replication times for some viruses and bacterial strains can delay confirmation of the initial diagnosis for more than a week. The most commonly used alternative method is the ELISA. However, cross-reactive antibodies against viruses, particularly when they are part of the same virus family, may confound the results of serologic tests and may lead to misinterpretation during the epidemiologic assessment in regions of the world where they are co-endemic [189]. For instance, among the flaviviruses, serological cross-reactivity between Zika virus and dengue virus confounds diagnosis of Zika virus infections in pregnant woman in regions where Dengue virus is also endemic [190]. Additionally, ELISA-detecting antibodies (IgG and IgM) that were produced against the virus do not identify the active infection or the virus particles. Because they can interact with different regions of the protein compared with antibodies, aptamers might be capable of distinguishing viruses that cannot be distinguished serologically [162]. Aptamers that have been reported to specifically bind flaviviruses and their protein products including Ebola [162], Zika [163], and dengue virus [160] should be tested for their abilities to distinguish these viruses.

The abilities to detect, identify, and quantify microbes and viruses and to identify virally infected cells are essential for their early diagnosis. An increasing number of aptamers have been isolated that bind specific microbes such as *Escherichia coli*, *Bacillus thuringiensis*, *Campylobacter jejuni*, *and Campylobacter coli* [191], various salmonella species, including *S. enteritidis* [72] and *S. enterica* [65], and staphylococcus species including *S. aureus* [79], *S. typhimurium* [67], and *S.*

enteritidis [192] (**Table 2**). In addition to the aptamers recognizing flaviviruses discussed in the previous paragraph, there are also aptamers that bind HIV-1 and hepatitis C virus (HCV) [188], among others (**Table 3**).

Aptasensors that have been developed using a number of the aptamers just mentioned hold the promise of prompt management of infections with a decreasing incidence of morbidity. Another possible application of aptasensors is in vaccine development. The costs and extended time associated with the assessment of vaccine concentrations by ELISA might be overcome by the application of aptasensors [193].

3.2 Future directions in clinics

Application of aptamer technology in the clinic has the potential of solving some stubborn diagnostic problems. Here we discuss some examples.

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and is one of the top ten causes of death worldwide [194]. The incidence of childhood TB is reported as a half a million cases with 74,000 deaths annually by the World Health Organization [195]. The most commonly used diagnostic tool for tuberculosis is the TB skin test. However, a false-positive test result in people vaccinated with the bacillus Calmette-Guerin (BCG) vaccine can be a confounding factor for diagnosis. Thus, the distinction of infection from disease, particularly in children, is still unclear. Additionally, microbiologic confirmation of bacteria in body fluids in childhood is difficult because of the poor bacillary count [195–197].

Cytomegalovirus (CMV) causes life-threatening infections in patients with solid organ transplantation, hematopoietic stem cell transplantation, and AIDS. Cytomegalovirus causes acute and latent infections and reactivates in immunosuppressed patients. Isolation and infection control procedures as well as proper management of VHFs commonly depend on an accurate diagnosis [198]. Current diagnostic tests targeting CMV DNA and CMV antigens are insufficient for discriminating acute and latent infections and detecting organ involvement. As a result, the results of these tests are frequently misinterpreted [199–201]. Viral hemorrhagic fevers (VHFs) are caused by a couple of viruses including *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, and *Flaviviridae*. Fulminant and fatal disease processes are the common features of the VHFs and diagnosing and distinguishing of VHF from other tropical diseases may be problematic because of the indiscriminatory symptoms [202].

An accurate and reliable diagnosis of these and other infections will provide for appropriate management and will decrease morbidity and mortality. Thus, development of fast microorganism-focused tests will provide rapid accurate diagnosis of infection and organ involvements. It seems inevitable that, in the near future, many aptamer-based methods/tools will provide for early diagnosis that will enable rapid initiation of optimal treatment regimens for viral and bacterial diseases.

4. Future perspective

First identified in 1890 [203], antibodies and their means of interaction with their antigens were already being extensively studied in the early twentieth century, and their structures were reported in the 1960s by Gerald Edelman [204]. Continuing studies of antibodies and the means of eliciting them have resulted in a detailed understanding of how they interact with their antigens. This knowledge combined with an expansive array of available antibodies motivates scientists to incorporate them into new diagnostic tools. Thus, antibody use dominates the 45 billion dollars global diagnostics market.

The more recently discovered aptamers are generally compared with antibodies due to their functional similarity. Since the discovery of aptamers in the 1990s, over a thousand studies have been conducted on applications of aptamers for diagnostics. Aptamers that specifically target biomarkers and bacterial or viral virulence factors such as surface glycoproteins or secreted proteins have been generated. These studies have demonstrated the range of targets that can be recognized by aptamers and the number of sensor platforms into which aptamers can be incorporated, many of which have been discussed in this chapter. However, the more structurally flexible aptamers are not as readily plugged into standard diagnostic assays as antibodies. In this burgeoning and yet immature field of aptasensors, there is still much to be learned about how to control aptamer behavior.

More systematic studies are needed to optimize selection methods, and more aptamers need to be characterized structurally. The biological matrix in which the analyte will be measured in the final application platform should be considered at the beginning of the selection so as to use buffer and ionic compositions during SELEX that resemble the target matrix. Maturation of aptamers to increase specificity for their target analyte in the appropriate matrix and for effective performance in the chosen reporter platform is also extremely important.

Aptamers offer the allure of easier production, ease of chemical modification, smaller size, reusability, stability even at high temperatures, low cost, and a long shelf life. A variety of chemical modifications further enhance aptamer stability. A significant advantage over antibody-based assays is that aptamers can be reused for many cycles without losing potency with the analyte being removed between each cycle by heating or other means. These features hold promise for the continued incorporation of aptamers into various sensor platforms and for the further development and eventual commercial application of aptasensors for diagnostics.

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Conflict of interest

MNH is the owner of Aptalogic Inc. located in Ames, IA, USA.

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